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Preliminary evaluation of physical and chemical properties of *Piper guineense* and *Xylopia aethiopica* seed oils

*Ogbonna, A. C., Abuajah, C. I. and Hart, E. B.

Department of Food Science and Technology, University of Uyo, P.M.B 1017, Uyo, Nigeria

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Abstract

Piper guineense and *Xylopia aethiopica* are commonly used as spices and traditional medicine in Southern Nigeria. The nutritional and health potential of their seed oils were evaluated by the study using standard methods. Values of all measured properties of the oil samples, except for peroxide and pH, were significantly ($p < 0.05$) different. The peroxide value of *Piper guineense* oil sample was 0.92 ± 0.01 mEq/Kg while *Xylopia aethiopica* oil gave a peroxide value of 13.35 ± 0.05 mEq/Kg which suggested that some level of oxidation might have occurred in the *Xylopia aethiopica* oil between the time of extraction and analysis. The iodine, saponification and ester values of *Piper guineense* oil sample were 13.66 ±0.70 gI_2/100g; 13.18 ± 0.98 mgKOH/g and 154.97 ±1.10 mgKOH/g whereas those of *Xylopia aethiopica* oil were 75.30 ± 1.83 gI_2/100g; 171.82 ± 5.07 mgKOH/g and 108.70 ± 1.50 mgKOH/g, respectively. However, their acid values (21.48 ± 1.21 mgKOH/g for *Piper guineense* and 16.85 ± 1.64 mgKOH/g for *Xylopia aethiopica*) and free fatty acid values (10.74 ± 0.82 mgKOH/g for *Piper guineense* and 8.43 ± 0.41 mgKOH/g for *Xylopia aethiopica*) were low whereas their unsaponifiable matter values were correspondingly high (69.16 ± 1.03% for *Piper guineense* and 19.16 ± 0.92% for *Xylopia aethiopica*) when compared to those of some popular seed oils. Their pH values at 25ºC were mildly acidic (5.76 ± 0.08 for *Piper guineense* and 5.10 ± 0.07 for *Xylopia aethiopica*). Some level of antioxidant activity was observed in the two oil samples. Thus, the oil samples investigated could be classified as non-drying.

Introduction

Lipids play important roles in both nutritional and industrial purposes globally (Idouraine *et al*., 1996). No oil from a single source has been found to be suitable for all purposes, thus the need to study their constituents (Bello *et al*., 2011). The characteristics of oils from various sources differ as a result of their fatty acid compositions (Ozcan *et al*., 2010; Bello *et al*., 2011).

However, consumers are constantly becoming aware of the health benefits associated with the intake of a wide variety of seed oils. Poly- and mono-unsaturates have been reported to be essential for normal growth and development as well as play important roles in the prevention and treatment of coronary artery disease, hypertension, diabetes, arthritis, other inflammatory and autoimmune disorders (Wang, 2004).

The seeds and leaves of *Piper guineense* and *Xylopia aethiopica* are consumed in Southern Nigeria and some parts of West Africa because of their spicy aroma. They are known to contain oils (Ayedoun *et al*., 1996; Jirovetz *et al*., 1997; N'dri Konan *et al*., 2009). Their uses in traditional medicinal practices in Africa and beyond is well documented (Ekanem and Obiekezie, 2000; Ekanem *et al*., 2004; N'dri *et al*., 2009; Nwinyi *et al*., 2009; Ezekwesili *et al*., 2010; Acquaye *et al*., 2011). In some parts of West Africa, hot extracts of the seeds and leaves of *Piper guineense* and *Xylopia aethiopica* serve as tonic for women after childbirth to enhance uterine contraction, expulsion of after-birth and draining of excess fluid to control weight, as well as acting as an aphrodisiac (Mbongue *et al*., 2005; Abolaji *et al*., 2007; Ekanem *et al*., 2010). Their medicinal properties is a function of phytochemicals which have been implicated in disease prevention as well as having antiparasitic effects on microbes (Okwu, 2003; Angioni *et al*., 2004; Okigbo and Igwe, 2007; Abolaji *et al*., 2007; Fattouch *et al*., 2007; Nwinyi *et al*., 2009; Ezekwesili *et al*., 2010; Bassey *et al*., 2011).

Piper guineense is a tropical plant which belongs to the family Piperaceae and has more than 700 species (Nwinyi *et al*., 2009). The plant is a climbing vine that can grow up to 20m in length and bears pepperish berry fruits (Figure 1). In Nigeria, it is known by various vernacular names such as

*Corresponding author.
Email: *acogbonna@yahoo.com*

Figure 1. Fresh seeds of *Piper guineense*

Figure 2. Dried fruit hulls of *Xylopia aethiopica*

uziza in Igbo; *iyere* in Yoruba and *odusa* in Ibibio (Nwinyi *et al*., 2009; Ekanem *et al*., 2010). Similarly, *Xylopia aethiopica* is a slim, tall tree of about 60 - 70 cm in diameter and can reach up to 15-30cm tall (Acquaye *et al*., 2011). Its fruit hull (Figure 2) which is aromatic is rather small and looks like twisted bean-pods (Acquaye *et al*., 2011). It belongs to the family Annonaceae, genus *Xylopia* and has numerous species which include *aethiopica* (Hyde *et al*., 2012). It is commonly known as West African pepper (Tairu *et al.,* 1999). According to Abolaji *et al.* (2007) and Bassey *et al.* (2011), it is called different vernacular names by different tribes in Nigeria including *ata* in Ibibio, *eeru* in Yoruba and *uda* in Igbo. In this paper, the nutritional and health status of *Piper guineense* and *Xylopia aethiopica* seed oils through the evaluation of their physicochemical properties is reported.

Materials and Methods

Source and preparation of samples

Some matured seeds of *Xylopia aethiopica* and *Piper guineense* were purchased from a local market in Akwa Ibom state, Nigeria. The seeds were identified and certified by the Department of Botany and Ecological Studies, University of Uyo, Nigeria. The choice of these plants and the parts of the plants used for the work was based on their common usage as food spices in the South-East and South-South areas of Nigeria. The seeds were cleaned; sun-dried and milled to pass 0.21 mm sieve (mesh number 65 - Tyler approx. or 70 - U.S. approx.) in a Laboratory Sieve Shaker (Norstone Inc., Bridgeport PA, U.S.A), with a Q-link Electric Blender (Model QBL-20L40, Made in China). All the chemicals and reagents used for this study were of analytical grade.

Extraction and analysis of oil samples

Fifty grams each, of the ground dry seeds of *Xylpoia aethiopica* and *Piper guineense*, was wrapped with a whatman filter paper and extracted using n-hexane in a reflux soxhlet extractor for 9 hours. The solvent was evaporated to dryness in an oven at 60ºC and the recovered oil was cooled in a dessicator. The oils recovered were stored in air-tight screw-capped flint-coloured glass bottles (to avoid photo-oxidation). The yield of the seed oil samples of *Xylopia aethiopica* and *Piper guineense* was determined gravimetrically (the ratio of the weight of oil samples recovered to the weight of the milled sample used in the extraction expressed in percent). Other physicochemical properties (specific gravity, iodine value, saponification value, unsaponifiable matter, pH, peroxide value, ester value and acid value) were determined according to standard procedures of AOAC (1990). The free fatty acid (FFA) was calculated from the relation: 1 unit of acid value = 2 × FFA% (Onwuka, 2005). The antioxidant property of the oil samples was evaluated by blending known volumes (1000 mL) of coconut oil, crudely extracted from matured kernels of *Cocos nucifera* according to the method proposed by Ambar and Sri (2010), with each of *Xylopia aethiopica* and *Piper guineense* oil samples at a ratio of 50:1 and storing them for four weeks in an enclosed chamber. An unblended sample of coconut oil without such treatment was set up as a control. The blends as well as the control were evaluated prior to storage and subsequently every week during the storage period for oxidative rancidity through the measurement of peroxide value. Each experimental method was replicated three times.

Statistical analysis

The standard deviation of data generated was calculated. The data were statistically analyzed using a one-way Analysis of variance (ANOVA). Least significant difference (LSD) was applied to separate and show means that differed significantly. Significant difference was accepted at $p < 0.05$ (Ubom, 2004).

Results and Discussion

Physicochemical properties

The results of the physico-chemical properties of *Piper guineense* and *Xylopia aethiopica* oil samples are shown on Figure 3. *Piper guineense* seed oil was warm to touch whereas *Xylopia aethiopica* was a cold liquid at room temperature of 29 ± 1.0ºC. The mean yield of the two oil samples (37.82% for *Piper guineense* and 25.64% for *Xylopia aethiopic*) from the same extraction process were significantly (p <

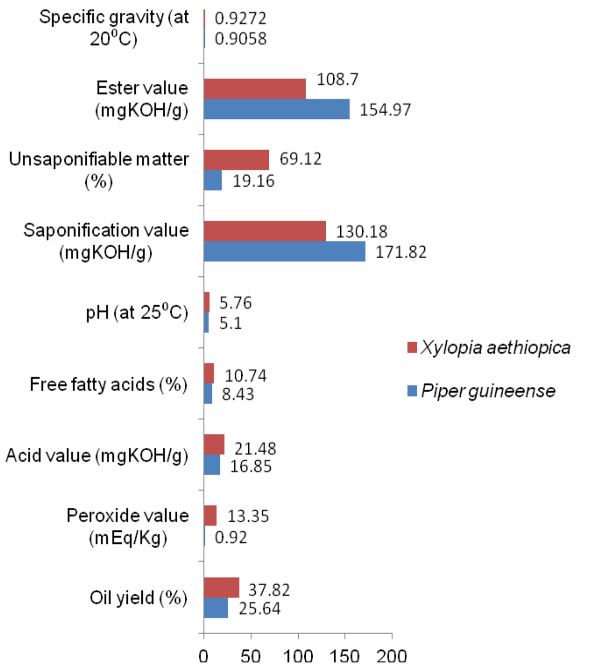
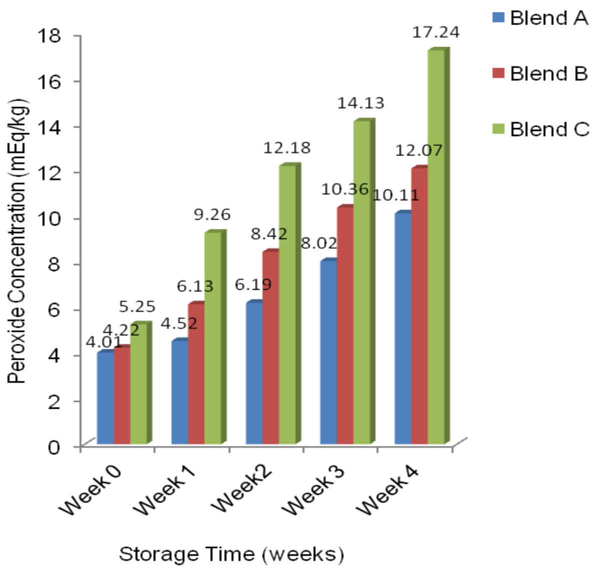


Figure 3. Physico-chemical characterization of *Piper guineense* and *Xylopia aethiopica* oil samples



Legend Description:
Blend A: Coconut oil sample + *Piper guineense* oil.
Blend B: Coconut oil sample + *Xylopia aethiopica* oil.
Blend C: Coconut oil sample alone (control).

Figure 4. Peroxide values of blends and control samples

0.05) different. The results were within the range of 30% yield reported for *Piper guineense* dried fruit powder extracted in 90% methanol by Miegel *et al.* (2007) but at variance with 3.57% and 4.68% reported for essential oils of *Xylopia aethiopica* by Lamaty *et al.* (1987).

Peroxide value of *Piper guineense* oil was 0.92 ± 0.01% indicating that it was stable to oxidation after two days between extraction and analysis. However, the peroxide value for *Xylopia aethiopica* was 13.35 ± 0.05 mEq/Kg. This was higher than the maximum limit of 10 mEqKOH/Kg set by Codex Alimentarius Commission for nuts and seed oils (Abayeh *et al.*, 1998; FAO/WHO, 2001) suggesting that some level of oxidation might have occurred in the oil before analysis. Peroxide value is an index of rancidity, thus low peroxide value indicates resistance of oil to peroxidation during storage (Bello *et al.*, 2011).

The iodine values of *Piper guineense* oil (13.66 ± 0.70 gI_2/100g) and *Xylopia aethiopica* (75.30 ± 1.83 gI_2/100g) were significantly ($p < 0.05$) different. The iodine value of *Xylopia aethiopica* oil indicated a higher degree of unsaturation than that of *Piper guineense.* Iodine value is a measure of the degree of unsaturation in oil and could be used to quantify the amount of double bonds present in the oil which in turn reflects its susceptibility to oxidation (Bello *et al.*, 2011). From the iodine values of the oil samples, they could be classified as non-drying oils (Fernando and Akujobi, 1987). Non-drying oil is that which does not harden when exposed to air. This is opposed to drying oil which hardens completely or semi-drying oil which partially hardens when exposed to air. Oils with I_2 number less than 115 gI_2/100g are considered non-drying and those above 130 gI_2/100g are considered drying. In between are the semi-drying types. Higher iodine value in oils is desirable nutritionally. However, the higher the iodine value, the less stable the oil and the more vulnerable it is to oxidation and production of free radicals.

The acid and free fatty acid values of the oil samples (21.48 ± 1.21 mgKOH/g and 10.74 ± 0.82% for *Piper guineense* and 16.85 ± 1.64 mgKOH/g and 8.43 ± 0.41% *Xylopia aethiopica* oils, respectively) were significantly ($p < 0.05$) different. The acid value is an indicator of the degree of edibility of oil. This degree of edibility is generally considered to be inversely proportional to its free fatty acids content (Ali *et al*., 2008; Bachheti *et al*., 2012). The lower the free fatty acid level, the better the quality of the oil (Balley, 1982). Thus, the oil samples investigated were edible and suitable for nutritional purposes. Globally, current dietary trends are geared towards assisting consumers to choose low saturated fat diets that are beneficial for cardiovascular health (FAO, 1994).

The pH values of the oil samples (5.76 ± 0.08 for *Piper guineense* and 5.10 ± 0.07 for *Xylopia aethiopica*) were mildly acidic at ambient temperature. However, the values showed no significant ($p < 0.05$) difference.

The saponification values of *Piper guineense* (130.18 ± 0.98 mgKOH/g) and *Xylopia aethiopica*

(171.82 ± 5.07 mgKOH/g) oils was high and significantly ($p < 0.05$) different. High saponification value indicates the presence of fatty acids with higher number of carbon atoms and vice-versa. Saponification value is an indicator of the average molecular weight and, hence, chain length of a lipid (Ardabili *et al.*, 2011). Hence, the results suggested that *Piper guineense* and *Xylopia aethiopica* oil samples have high molecular weight respectively.

This study also revealed that the oil samples contained significantly ($p < 0.05$) high levels of unsaponifiable matter (69.16 1.03% for *Piper guineense* and 19.16 ± 0.92% for *Xylopia aethiopica*) compared to those of some popular seed oils such as 0.8% for cocoa butter and 0.64% for cashew nut oil (Ojeh, 1985); 13.6% for *Mucuna pruriens* and 3.1% for *Urena lobota* seed oils (Ahmad *et al.*, 1978) and 1.14% for *Hura crepitans* (Umoren *et al.*, 2001). The unsaponifiable matter in oils are a variety of non-glyceridic bioactive substances containing variable mixtures of hydrocarbons, aldehydes, ketones, alcohols, sterols, pigments and fat-soluble vitamins that may occur naturally or may be formed during processing or degradation of oils (Badifu, 1991). This result probably indicates that *Piper guineense* and *Xylopia aethiopica* may contain high levels of bioactive components and phytochemicals which possess the capacity to prevent and cure diseases resulting to health benefits and promotion of well-being (Wildman, 2001; Swanson, 2003). This will require further works to confirm.

The ester values (154.97 ± 1.10 mgKOH/g for *Piper guineense* and 108.7 mgKOH/g for *Xylopia aethiopica*) of the oil samples were calculated as the differences between their saponification and acid values. The ester values of the oil samples were significantly ($p < 0.05$) different and comparable to 190 mgKOH/g for cotton seed and 254 mgKOH/g for coconut oils (Woollat, 1985). The implication of esters in oils is that esters being aromatic in nature make oil samples with significant ester values useful in the production of cosmetics and other aromatic products.

The *Piper guineense* and *Xylopia aethiopica* oil samples had significantly different ($p < 0.05$) specific gravities of 0.9058 ± 0.02 and 0.9272 ± 0.01, respectively. These values are comparable to 0.908 for *Hura crepitans* oil (Umoren *et al.*, 2001), 0.939 for Neem seed and 0.918 for groundnut oils (Akpan, 1999). Specific gravity is used to assess the purity of oil (Ojeh, 1985).

Antioxidant activity

The antioxidant activity of the oil samples based on peroxide values of their blends measured during four weeks storage is shown in Figure 4. Rancidification was observed in all the samples during the storage period as shown by the significantly ($p < 0.05$) different peroxide levels measured. However, the different rates at which the blends and control were oxidized indicated some level of antioxidant activity of the two oil samples investigated. The seed oil of *Piper guineense* showed greater capacity to retard oxidation. This could have been as a result of its low I_2 value which is indicative of a higher saturated acid content than the oil sample of *Xylopia aethiopica* seeds. It has been shown that oils become rancid when their peroxide value ranges from 20.0 to 40.0 $mEqO_2$/Kg (Ajayi *et al*., 2006). Biochemically, antioxidants retard oxidative reactions by intercepting charged free radicals and reactive oxygen species (Brennam *et al*., 2000; Crandell and Duren, 2007; Abuajah *et al.*, 2014).

Conclusion

The physicochemical properties of the seed oils of *Piper guineense* and *Xylopia aethiopica* from this study suggest that they could be classified as non-drying in nature, edible and nutritionally healthy. However, this preliminary study indicated that the oils possess some level of antioxidant activity by slowing the rate of rancidification of their blends during storage. This will be further confirmed in a subsequent paper at the end of the broader study. Blend A (*Piper guineense* oil + coconut oil) had the least rancidification whereas the control (Coconut oil alone) was most rancidified.

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